

DOCKET NO.: 0690-0122PUS1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF : KOBER et al.
SERIAL NO. : 10/525,008
FILED : September 8, 2005
FOR : AGENTS CONTAINING CARBOXYLIC ACID
AND THE USE OF THE SAME IN PLANT
CULTIVATION

DECLARATION UNDER 37 C.F.R. §1.132

COMMISSIONER OF PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

SIR:

Now comes Jan Willmann who deposes and states:

1. I am a graduate of the University of Bremen, Germany, and received my doctorate degree in the year 2007.
2. I have been working for more than 5 years as an NMR specialist in the field of organic chemistry, since 2009 in the crop protection division at BASF SE.
3. I was asked to investigate by NMR whether or not metconazole is protonated by propionic or sulfuric acid.
4. The following experiments were carried under my supervising regime in a GLP (Good Laboratory Practice) certified laboratory. The Certificate is shown in figure 3 which is attached hereto as Annex II.
5. NMR measurements were done on a Varian Unity INOVA 600 MHz NMR-Spectrometer equipped with an idhfx probe. VnmrJ (version 2.2 revision D) was used as data processing system.

6. GLP-certified 1*H*-1,2,4-triazole and metconazole samples were taken from the inhouse reference material team. All sample solutions were prepared as follows:

40.0 mg of metconazole (Reg.No. 4056343; $M=319,8$ g/mol) were dissolved in 1.5 mL of deuterated DMSO(-d₆) (metconazole stock solution). 29.0 mg of unsubstituted 1*H*-1,2,4-triazole (Reg.No.87084; $M=69$ g/mol) were dissolved in 5.0 mL of deuterated DMSO(-d₆) (1*H*-1,2,4-triazole stock solution). 24.5 mg of propionic acid ($M=74$ g/mol) were dissolved in 1.5 mL of deuterated DMSO(-d₆) (propionic acid stock solution). 36.5 mg of sulfuric acid (98% purity; $M=98$ g/mol) were dissolved in 1.5 mL of deuterated DMSO(-d₆) (sulfuric acid stock solution).

For each NMR measurement, 0.6 mL (50 μ mol) of the metconazole stock solution or 0.6 mL (50.4 μ mol) of the 1*H*-1,2,4-triazole stock solution were transferred to a NMR glass tube (control samples). Test samples were prepared by adding either 0.23 mL (50.7 μ mol) or 0.46 mL (101.4 μ mol) of the propionic acid stock solution or 0.2 mL (49.7 μ mol) of the sulfuric acid stock solution to the NMR tubes containing the metconazole or 1*H*-1,2,4-triazole stock solution.

The composition of all samples is summarized in table 1:

Table 1:

Sample No.	Metconazole 0.6 mL (50.4 μ mol)	1 <i>H</i> -1,2,4- Triazole 0.6 mL (50.4 μ mol)	Propionic acid 0.23 mL (50.7 μ mol)	Propionic acid 0.46 mL (101.4 μ mol)	Sulfuric acid 0.2 mL (49.7 μ mol)
1a	X	-	-	-	-
1b	X	-	X	-	-
1c	X	-	-	X	-
2a	X	-	-	-	X
3a	-	X	-	-	-
3b	-	X	X	-	-
3c	-	X	-	X	-
4a	-	X	-	-	X

7. ¹H- and ¹H-¹⁵N-HMBC-NMR measurements were done of the metconazole stock solution (sample 1a), the equimolar metconazole / propionic acid solution (sample 1b), the 2:1

(mol:mol) propionic acid / metconazole solution (sample 1c), and the metconazole / sulfuric acid solution (sample 2a). ^1H -NMR measurements were done of the 1*H*-1,2,4-triazole solution (sample 3a), the equimolar 1*H*-1,2,4- triazole / propionic acid solution (sample 3b), the 2:1 (mol:mol) propionic acid / 1*H*-1,2,4-triazole solution (sample 3c), and the 1*H*-1,2,4-triazole / sulfuric acid solution (sample 4a). All ^1H chemical shifts were referenced to the DMSO signal.

8. The ^1H - and ^{15}N -HMBC-NMR spectra recorded for samples 1a, 1b, 1c and 2a are shown in Figures 1a- ^1H , 1a- ^{15}N , 1b- ^1H , 1b- ^{15}N , 1c- ^1H , 1c- ^{15}N , 2a- ^1H , 2a- ^{15}N which are attached hereto as Annex I. All results are summarized in table 2:

Table 2:

Sample No.	Ingredients / mixtures	^1H - chemical shifts [ppm]		^{15}N - chemical shift [ppm]		
		H^3	H^5	N^1	N^2	N^4
1a	Metconazole in DMSO(-d6)	8.0	8.5	198.0	113.5	152.5
1b	Metconazole in DMSO(-d6) + 1 mol eq. propionic acid	8.0	8.5	197.9	113.6	152.2
1c	Metconazole in DMSO(-d6) + 2 mol eq. propionic acid	8.0	8.5	198.0	113.5	152.4
2a	Metconazole in DMSO(-d6) + 1 mol eq. sulfuric acid	8.4	9.0	198.2	115.6	126.1
3a	1 <i>H</i> -1,2,4-triazole in DMSO(-d6)	8.0	8.5	-	-	-
3b	1 <i>H</i> -1,2,4-triazole in DMSO(-d6) + 1 mol eq. propionic acid	8.0	8.5	-	-	-
3c	1 <i>H</i> -1,2,4-triazole in DMSO(-d6) + 2 mol eq. propionic acid	8.0	8.5	-	-	-
3d	1 <i>H</i> -1,2,4-triazole in DMSO(-d6) + 1 mol eq. sulfuric acid	9.1	9.1	-	-	-

9. As shown in table 2, the ^1H - and ^{15}N -NMR chemical shifts observed for samples 1a to 1c were identical. Accordingly, there was no indication that metconazole was protonated by the addition of 1 or 2 molar equivalents of propionic acid. In contrast, a strong shift of the ^{15}N signals,

especially for nitrogen N⁴ which is shifted from 152.4 to 126.6 ppm, and a slight shift of the proton signals (0.4 and 0.5 ppm) were observed when 1 molar equivalent of concentrated sulfuric acid was added to metconazole (sample 2a). This is a strong indication that especially nitrogen N⁴ of metconazole was protonated by the addition of sulfuric acid.

Analogous results were obtained with ¹H-NMR measurements of unsubstituted 1H-1,2,4-triazole (samples 3a to 3c and 4a) which fully confirmed the results obtained for metconazole.

10. I conclude that the NMR results obtained in this study corroborate that while metconazole is protonated by sulfuric acid, it is not protonated by propionic acid.

11. Further deponent saith not.

Limburgerhof, Germany, 07.07.2010



(Jan Willmann)

Annex I: Figures 1a-¹H, 1a-¹⁵N, 1b-¹H, 1b-¹⁵N, 1c-¹H, 1c-¹⁵N, 2a-¹H, 2a-¹⁵N
Annex II: Figure 3

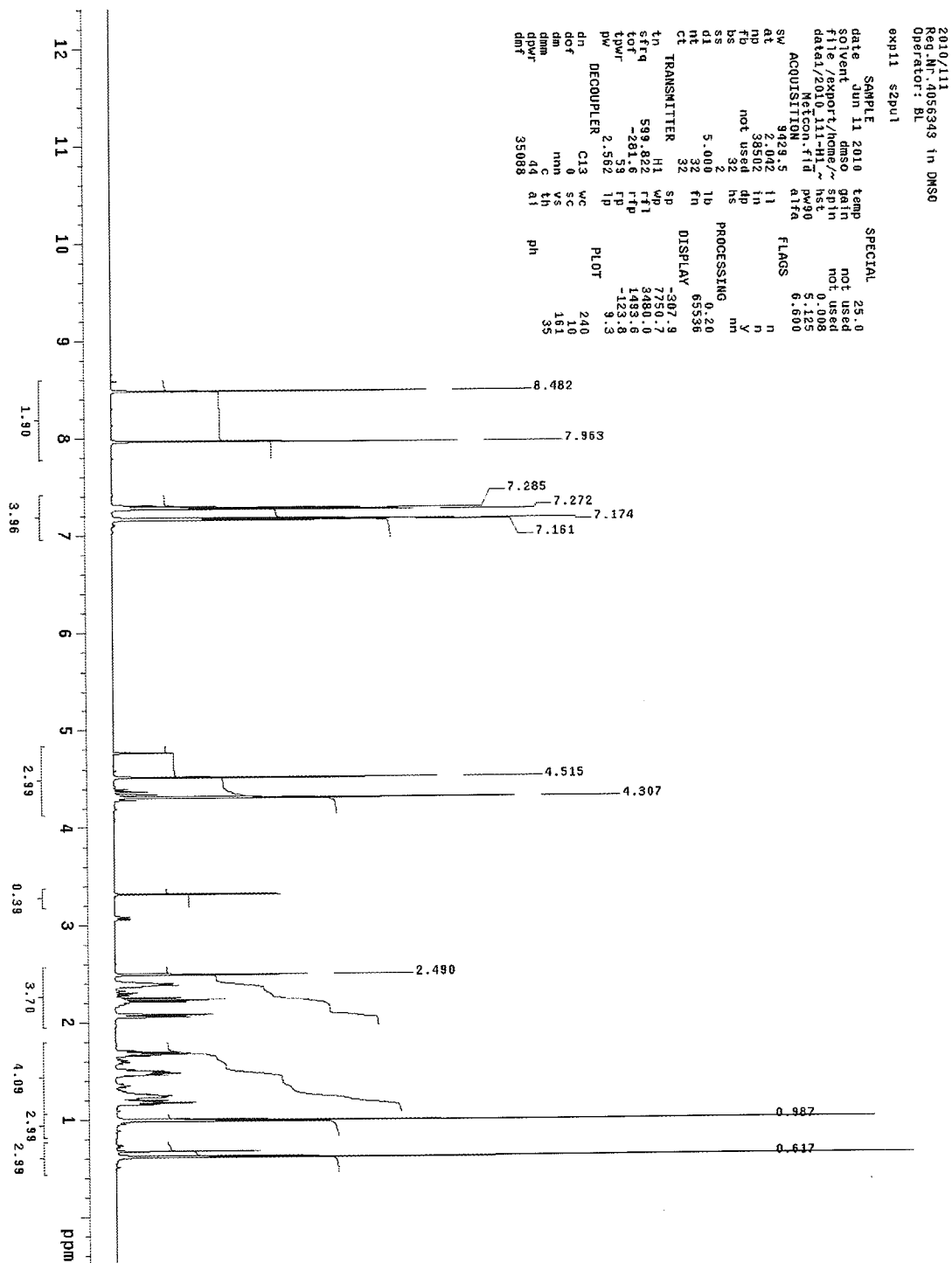
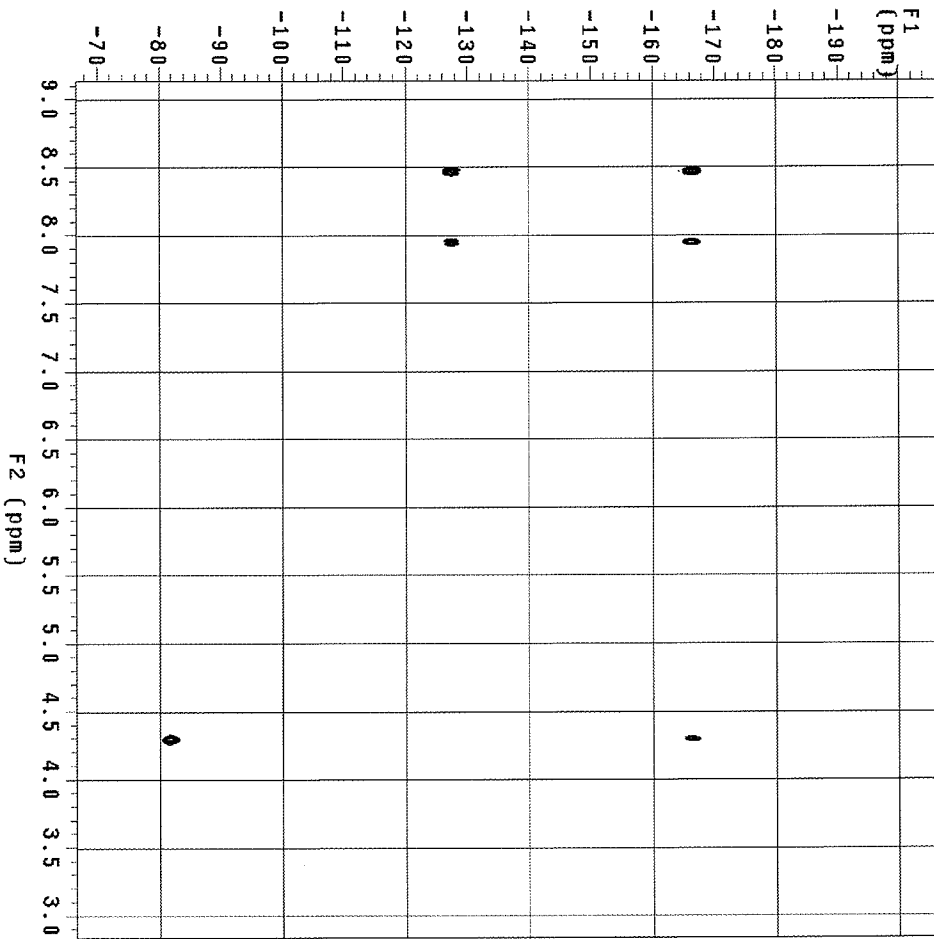
Figure 1a-¹H

Figure 1a-¹⁵N

2010/11/1
 Reg.NT:4056343 in DMSO
 Operator: BL
 exp12 Gmmbc

SAMPLE
 date Jun 11 2010
 solvent dmsc
 sample
 ACQUISITION
 sw 6460.5
 at 0.128
 np 1654
 fb not used
 ss 32
 d1 1.000
 nt 20 ACQUISITION 16
 sw1 17024.9
 nt 256
 phase PRESATURATION 0
 satmode mm
 satdly 0
 satfrq 499.8
 satpwr -13
 TRANSMITTER H1
 tn 599.822
 sfreq -750.6
 lof 59
 tpwr 5.125
 pw DECOUPLER M15
 dn 28110.6
 dof 200
 dmf mm
 dpmf mm
 dpmv1 0
 pmx 23.000
 j1xh 140.0
 jmxh 8.0

FLAGS
 hs
 sspul
 pfgf1g
 hsgiv1
 SPECIAL
 temp 25.0
 gain 42
 spin not used
 GRADIENTS
 g2iv11 4812
 g2iv13 962
 g13 0.001000
 g1ab 0.000500
 F2 PROCESSING
 sb 0.064
 sbc not used
 fn 4096
 F1 PROCESSING
 sb1 0.008
 sbc1 not used
 PROC1 2048
 DISPLAY
 sp 1702.8
 wp 3772.8
 sp1 -12915.5
 wp1 8462.6
 r1 981.7
 r1d 9588.3
 r1i1 -5005.4
 r1p1
 WC 130.0
 SC 10.0
 WC2 130.0
 SC2
 ts 1.64857e+06
 at CDC av 13



2010/111
Reg.Nr.4056343 in DMSO
equimolar mit Propionsäure
Operator: BL
exp16 Ghmbc

date	SAMPLE	Jun 11 2019	hs	flags	n
solvent	dmso	ssm1	PROG1	y	
sample	ACQUISITION	hsg1v1	SPECIAL	25.0	
sw	7107.3	temp		42	
at	0.128	gain		42	
np	1820	GRADIENTS	4812		
fb	not used				
ss	42	g2v1v3	0.001000		
sd	1.002	g2v1v3	962		
nt	16	g2v1v3	0.001000		
2D ACQUISITION	1.024	g13	0.001000		
sw1	17024.3	g5tab	0.000500		
ni	256	f2 PROCESSING			
phase	0	sbs	0.064		
PRESSATURATION	nmn	sbs	not used		
satmode	nmn	fn	not used		
satddy	493.8	fn	4935		
satfry	493.8	1 PROCESSING	0.007		
satpwr	13	sbs1	not used		
TRANSMITTER	H1	procl	ip		
tfq	599.822	fn1	2048		
tof	-750.6				
tpwr	5.125	DISPLAY			
pw	5	sp	1741.9		
DECOUPLER	M15	wd1	4056.9		
dn	M15	sp1	-12471.8		
dof	28110.6	wp1	9044.5		
dm	nmn	ft1	1305.1		
dmf	nmn	ft2	9605.0		
dmf2	20	ftp1	-5011.5		
PW1v1	60	PLOT			
pxw	28.000	wc	130.0		
HMBC	140.0	sc	10.0		
j1vh	8.0	wc2	130.0		
jvsh		sc2	0		
		vs	1.64857e+06		
		th	cdc av	13	

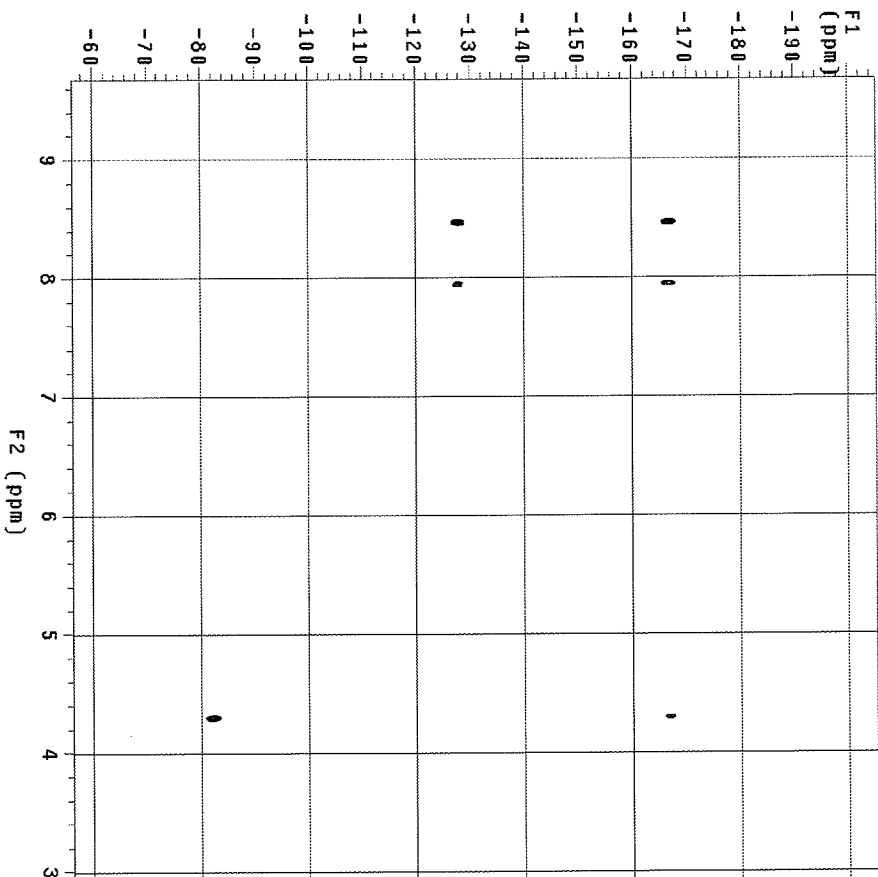


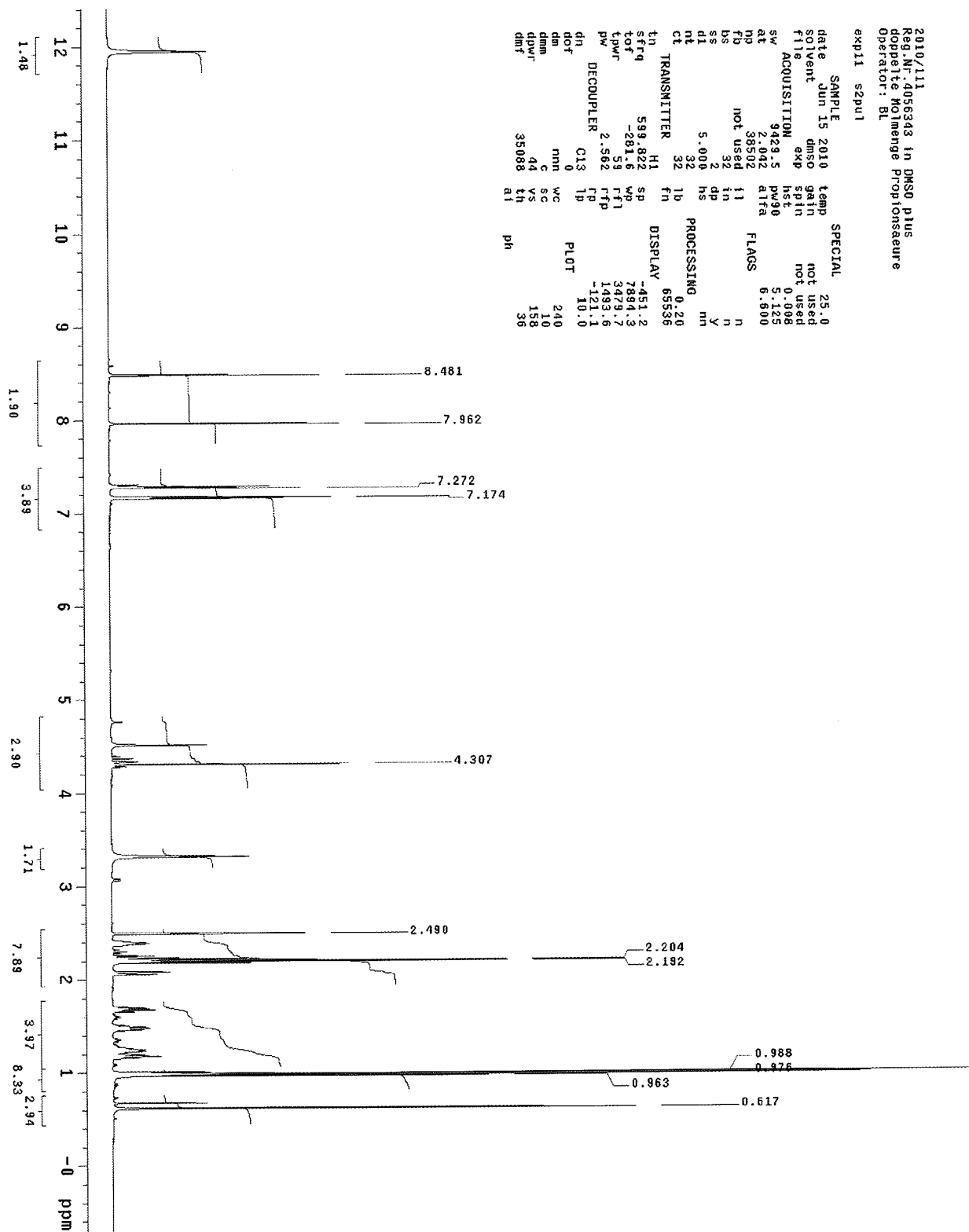
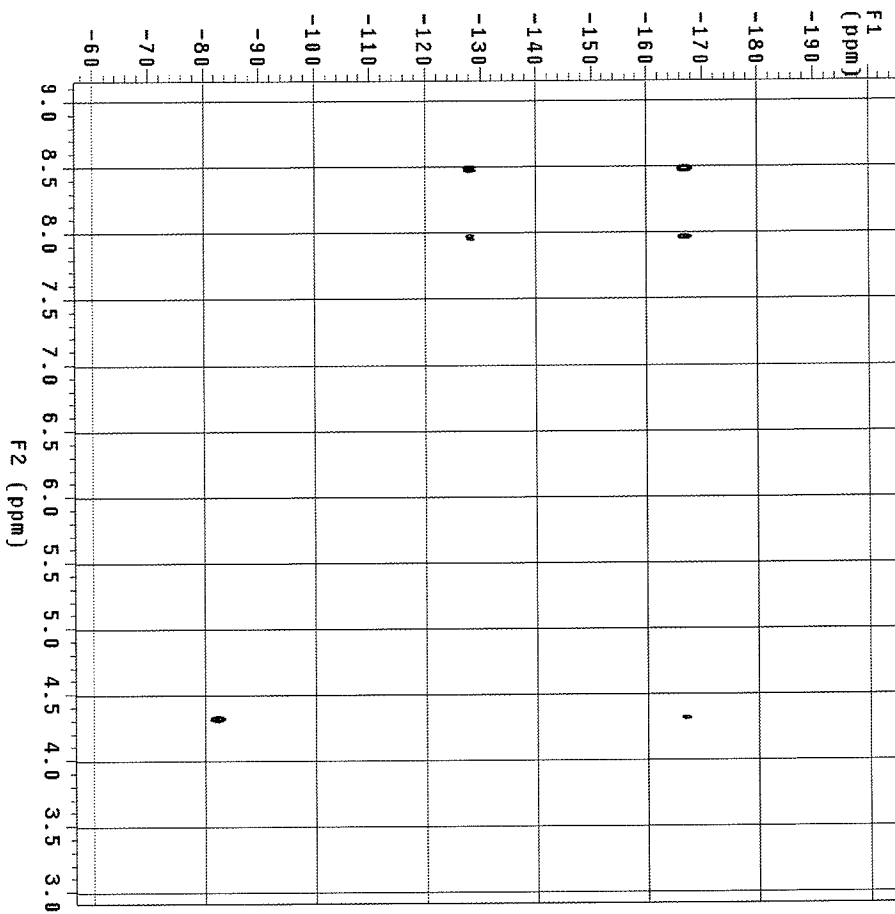
Figure 1c-¹H

Figure 1c-¹⁵N

2010/111
 Reg.Nr:4056343 in DMSO plus
 doppelte Molmenge Protonsaure
 Operator: BL
 exp20 Gmbc

SAMPLE		date		hs		flags	
solvent	dmso	date	time	hs	ssput	n	
sample	6460.5	Jun 15	2010	hs	ssput	n	
ACQUISITION	6460.5	hs	ssput	hs	ssput	n	
sw	0.128	temp	25.0	hs	ssput	n	
at	1654	gain	42	hs	ssput	n	
np	not used	spn	not used	hs	ssput	n	
fb	32	GRADIENTS	4812	hs	ssput	n	
ss	1.000	g2lv11	0.001000	hs	ssput	n	
dt	17024.9	g2lv13	0.001000	hs	ssput	n	
nt	17024.9	g2lv13	0.001000	hs	ssput	n	
2D ACQUISITION	17024.9	g2lv13	0.001000	hs	ssput	n	
sw	17024.9	g2lv13	0.001000	hs	ssput	n	
nt	17024.9	g2lv13	0.001000	hs	ssput	n	
phase	0	g2lv13	0.001000	hs	ssput	n	
PRESATURATION	0	g2lv13	0.001000	hs	ssput	n	
satmode	nm	g2lv13	0.001000	hs	ssput	n	
satdi	0	g2lv13	0.001000	hs	ssput	n	
satfrq	499.8	g2lv13	0.001000	hs	ssput	n	
satpwr	-13	g2lv13	0.001000	hs	ssput	n	
TRANSMITTER	H1	g2lv13	0.001000	hs	ssput	n	
tn	599.822	g2lv13	0.001000	hs	ssput	n	
strq	-750.8	g2lv13	0.001000	hs	ssput	n	
tot	5.125	g2lv13	0.001000	hs	ssput	n	
pw	5.125	g2lv13	0.001000	hs	ssput	n	
DECOUPLER	N15	g2lv13	0.001000	hs	ssput	n	
dn	28110.6	g2lv13	0.001000	hs	ssput	n	
dof	nm	g2lv13	0.001000	hs	ssput	n	
dof	200	g2lv13	0.001000	hs	ssput	n	
dof	0	g2lv13	0.001000	hs	ssput	n	
dof	0	g2lv13	0.001000	hs	ssput	n	
dmw	29.000	g2lv13	0.001000	hs	ssput	n	
dmw	60	g2lv13	0.001000	hs	ssput	n	
dmw	140.0	g2lv13	0.001000	hs	ssput	n	
dmw	8.0	g2lv13	0.001000	hs	ssput	n	
dmw	1.64857e+05	g2lv13	0.001000	hs	ssput	n	
dmw	4	g2lv13	0.001000	hs	ssput	n	



SAMPLE						SPECIAL
date	Jun 11 2010	temp	25.0			
solvent	dmsc	gain	not used			
file /export/home/	spin	used	not used			
data1[2010-11-hf	nst		0.008			
Schweifer's.HF	pvs9		5.125			
ACQUISITION	atfa	flags	6.000			
SW	9423.5					
at	2.002	i1				
np	38502	i2				
fb	not used	y				
ts	2	hs				
ss	2					
dl	5.000	fn				
cl	32	fp				
ci	32					
TRANSMITTER						
tn	H1	sp	-285.7	DISPLAY		
sfrq	539.482	rT1	772.8			
tof	-281.6	fTP	3479.7			
tpwr	59	FP	1493.6			
PW	2.552	lp	-111.3			
DECOUPLER						
dn	C13	wC	2400	PLOT		
dof	0	sc	1.79			
dm	nm	VS	2.9			
dmm	G	Th	4			
dprv	A	et	ph			
dmt	35068					

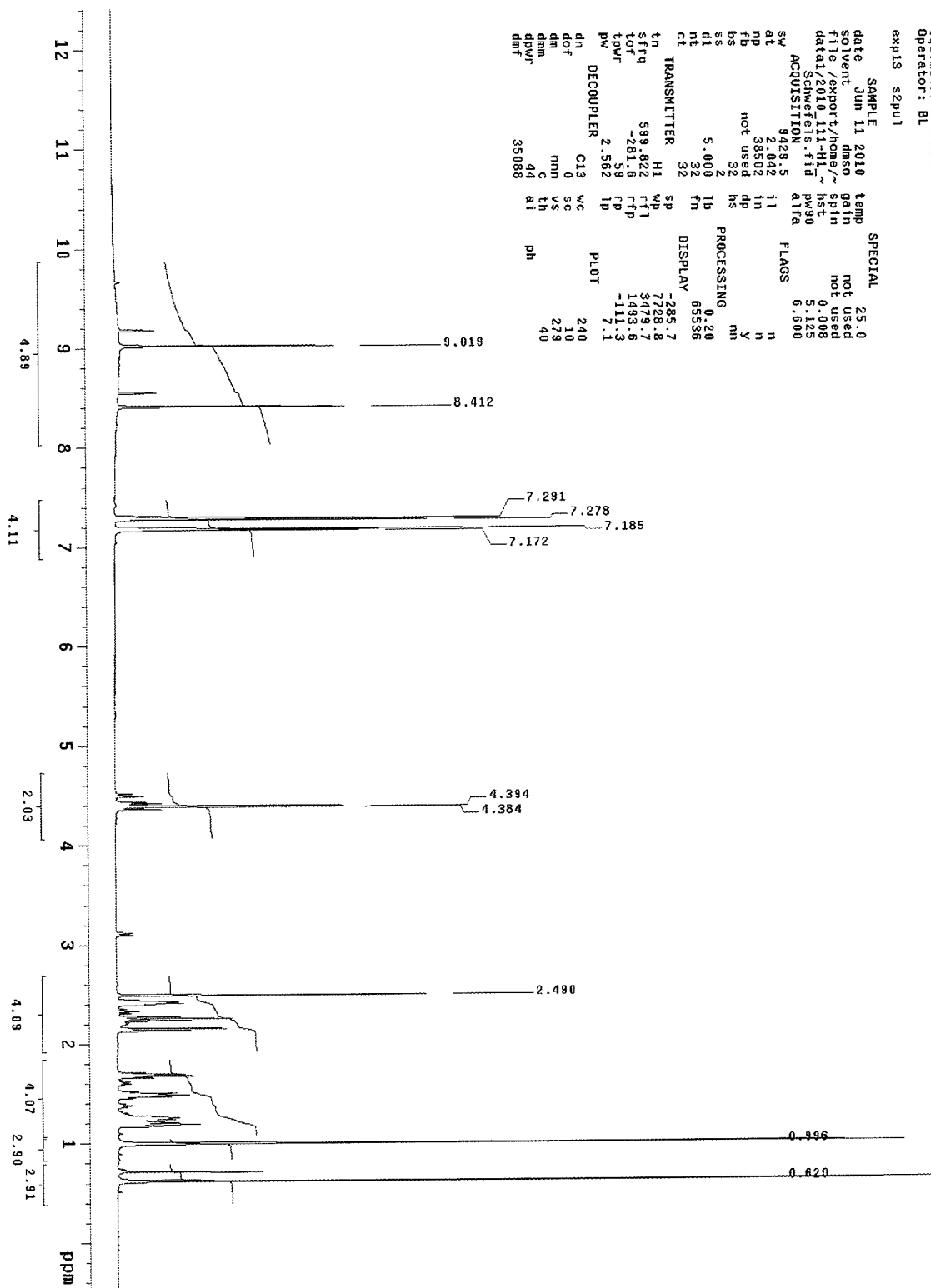
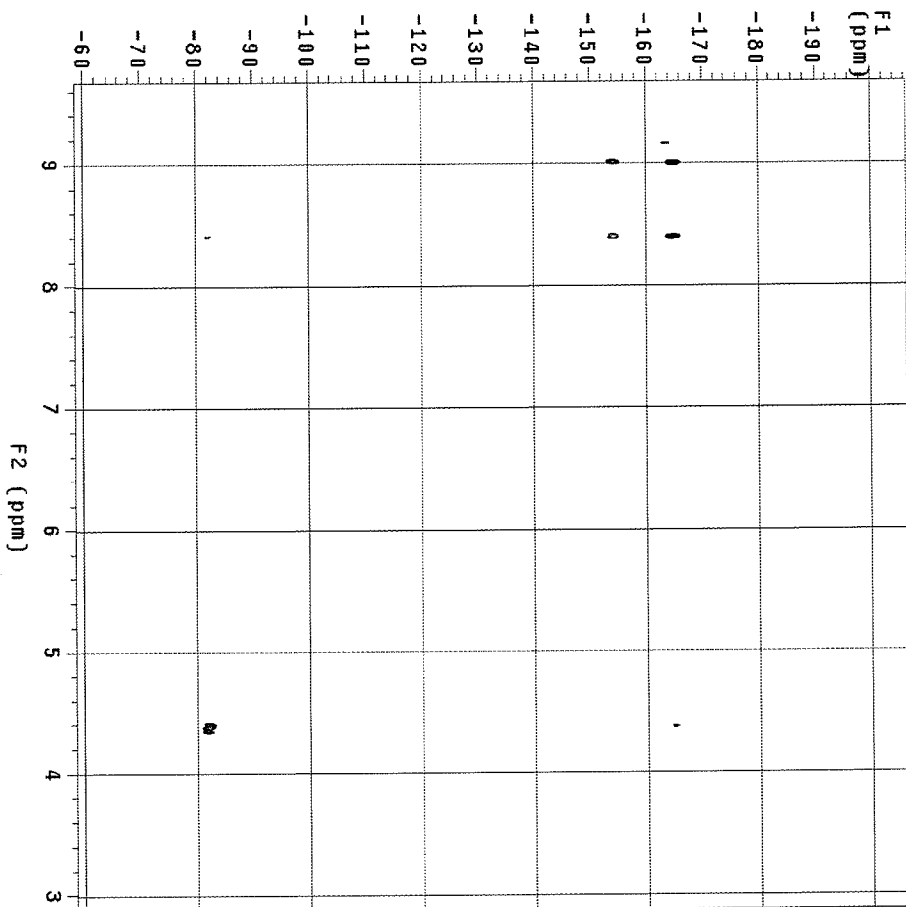


Figure 2a-¹⁵N

2010/11/11
 Reg.Nr.: 4056343 in DMSO
 Equimolar mit H2SO4
 Operator: BL
 exp14 Gmbhc

SAMPLE		FLAGS	
date	Jun 11 2010	hs	n
solvent	dmsc	sspu1	y
sample		ptg1g	4812
ACQUISITION		hsq1v1	SPECIAL
sw	7107.3	temp	25.0
at	0.125	gq1v1	not used
np	1640	sp1n	GRADIENTS
td	not used	gq1v1	4812
ss	not used	gq1v1	962
nt	1.000	gq1v1	0.001000
2D ACQUISITION		gq1v1	0.001000
sw1	17024.9	gq1v1	962
ni	256	gq1v1	0.000500
phase	0	gq1v1	0.064
PRESATURATION	0	gq1v1	not used
satmode	nm	gq1v1	not used
satdi1y	0	gq1v1	4096
satf1r	499.8	gq1v1	0.008
satpwr	-13	gq1v1	not used
TRANSMITTER	H1	gq1v1	2048
tn	599.822	gq1v1	1724.5
strq	-730.5	gq1v1	4074.2
tpwr	5.125	gq1v1	-12544.9
pw	28110.6	gq1v1	8878.0
DECOUPLER	N15	gq1v1	1305.1
dm	200	gq1v1	9629.8
dmp	0	gq1v1	-4993.3
dpwr	60	gq1v1	130.0
pwx1v1	29.000	gq1v1	10.0
hmhc	140.0	gq1v1	130.0
j1xh	8.0	gq1v1	1.64057e+06
jmxh		gq1v1	11



Annex II

Figure 3

Rheinland-Pfalz

Gute Laborpraxis / Good Laboratory Practice

GLP-Bescheinigung / Statement of GLP Compliance
(gem. / according to § 19 Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

Prüfeinrichtung / Test facility

BASF Aktiengesellschaft
BASF Agricultural Center Limburgerhof
Crop Protection Division
Ecology and Environmental Analytics
P.O. Box 120
D-67114 Limburgerhof

Prüfung nach Kategorien / Areas of Expertise
(gem. / according ChemVwV-GLP Nr. 5.3/OECD guidance)
1, 4, 5, 6, 7, 8

Datum der Inspektion / Date of Inspection
(Tag.Monat.Jahr / day.month year)
04. – 06. Oktober und 06. Dezember 2006

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

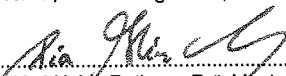
The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können. Eine erneute behördliche Überprüfung der Einhaltung der GLP-Grundsätze durch die Prüfeinrichtung ist so rechtzeitig zu beantragen, dass die Folgeinspektion spätestens vier Jahre nach dem Beginn der o.g. Inspektion stattfinden kann. Ohne diesen Antrag wird die Prüfeinrichtung nach Ablauf der Frist aus dem deutschen GLP-Überwachungsprogramm genommen und diese GLP-Bescheinigung verliert ihre Gültigkeit:

Based on the inspection report it can be confirmed, that the test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Verification of the compliance of the test facility with the Principles of the GLP has to be applied for in time to allow for a follow-up inspection to take place within four years after commencing the above mentioned inspection. Elapsing this term, the test facility will be taken out of the German GLP-Monitoring Programme and this GLP Certificate becomes invalid.

Unterschrift, Datum / Signature, Date

i.v. 
Dr.-Ing. Karl-Heinz Rother - Präsident -
(Name und Funktion der verantwortlichen Person / name and function of responsible person)



Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht
Kaiser-Friedrich-Straße 7
55116 Mainz

.....
(Name und Adresse der GLP-Überwachungsbehörde /
Name and address of the GLP Monitoring Authority)

Landesamt für
Umwelt, Wasserwirtschaft
und Gewerbeaufsicht

